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Dear Dr. Klieneberger-Nobel:

I was pleased to note (for my part) your preoccupation with E. coli K-12. I must admit that your letter did not give a very clear indidation of "it", that is your morphological observations, but perhaps you did not wish to commit yourself prematurely.

Your schedule for making up the medium appears to be quite satisfactory. We have used distilled water, but unless the tap water is incredibly contaminated with growth factors, it will work quite well instead. The effect of the tap water in stiffening the agar is probably its hardness (Ca and Mg), not the pH, for there is too much phosphate buffer in the medium for its final pH to be so readily modified. As a rule, we have not bothered to steam the sugar. The salts are weighed out and dissolved in one aliquot of water, the sugar and agar in another. These are then autoclaved separately, and mixed in equal proportions before the medium is poured. Some residual growth of the mutant cultures is to be expected, but the recombinant prototrophs should stand out as well-developed colonies against a barely visible background.

If we knew of any consistent way of increasing the rate of recombination, we should, of course be using it consistently. It has been reported that exposure of the 58-161 parent cells to small doses of ultraviolet light will increase their recombining propensity. We have not verified this for ourselves, as yet, but it would be worth trying.

I am not sure what controls will be possible. The same morphogenetic sequences that operate as between 58-161 x W-1177 undoubtedly occur within a K-12 culture, although without genetically meaningful consequences.

I am not sure what medium you are referring to in your question about alcalinization. The minimal medium would best be alcalinized by altering the proportions of the K_2HPO_4 : KH_2PO_4 to give the pH you want. I don't know what effect this will have-- (except that I must have done some experiments years ago, with no significant effect, so long as growth was not inhibited.) 11-Hour broth cultures should do well.

Our experiments with Streptomyces griseus are moving along. I would judge that heterokaryosis does occur, for different nutritional mutants combine to grow on minimal medium. However, sexual fusions if they occur at all are not as frequent as I would have expected if the aerial mycelium is regularly diploid. There are a number of possible complications, however, that prevent a definite conclusion just yet. Have your plans for a fellowship to this country matured to any extent?

Yours sincerely,

Joshua Lederberg